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STANDARDIZING THE CONDITIONS FOR PERFORMANCE OF IMMUNO--ELECTROPHORETIC EXPERIMENTS WITH TOBACCO STREAK ILARVIRUS PARTICLES

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The 0.016 M natrium phosphate pH 7.0 as a gel-buffer, of the two-fold molarity in the LKB-Gelman 6800 A—1 apparatus vessels, ensured the optimal immuno-electrophoretic patterns of tobacco streak ilarvirus (TSV/Cle) particles when a constant current of 4 mA and a voltage of 20 V/cm gel were applied for 4 h at 2°C. The conditions prooved adequate in demonstrating the electrophoretic heterogeneity of unfixed TSV particles.

Introduction

Although electrophoresis has been considerably less used in the characterisation of intact virus particles than of virus protein and nucleic acid, there are numerous publications describing this aspect of its application (Bancroft 1962, Niblett et Semancik 1969, Gellen *et al.* 1972, 1973, Hollings et Stone 1975, Lot et Kaper 1976, Diaz-Ruiz et al. 1979, Hanada 1984, Kunze et al. 1984, Musil et Galo 1984, 1986, Rana et al. 1987, etc; cf. Van Regenmortel 1982, Krajačić 1989). Since the charge of virus particles is one of the characteristics dependent on structure of capsid, electrophoretic analysis can be a satisfactory method in detection, identification and grouping of plant viruses, especially within their particular groups and subgroups. It can also imply the precursor-product relation, etc.

According to Van Regenmortel (1982) in immuno-electrophoresis alkaline buffers of pH 7.5—8.6 are mostly used. In such conditions proteins and viruses are negatively charged and move toward the anode.

The anomaly is the cathodic mobility of the negatively charged molecules which arises under the influence of electro-endosmosis (Hjerten 1961; *cf.* Andrews 1988). Although in most cases the buffers of an ionic strength between 0.025—0.05 M are used along with a field strength of 2—6 V/cm and electrophoresis duration of 1—3 hours, the precise voltage, amperage and length of time needed must be determined empirically for each particular system in accordance with buffer used and antigen being analysed.

The paper presents some experiments for standardizing the conditions needed for immuno-electrophoretic experiments with tobacco streak ilarvirus (TSV) particles by application of phosphate buffer.

Materials and Methods

Virus isolate and purification. The Cle strain of TSV was maintained in *Chenopodium quinoa* Wild. in a shaded greenhouse at 18—25°C (Rana et al. 1987). The virus was partially purified by modified procedure for elm mottle ilarvirus described by Jones and Mayo (1973). Inoculated and systemically infected leaves, harvested 8 days after inoculation, were homogenized (1 g/1 ml) in neutral 0.066 M sodium phosphate (Sörensen's) buffer which included 0.02 M 2-mercaptoethanol. Following clarification with 8.5% (v/v) n-butanol, the virus was further purified and concentrated by two cycles of differential centrifugation, by pelleting in a Beckman Spinco Model L centrifuge at 90 000 g for 90 min. The virus pellets were resuspended in neutral 0.01 M phosphate buffer.

Immuno-electrophoresis. Immuno-electrophoresis experiments were done using a LKB-Gelman 6800 A-1 microelectrophoresis apparatus in 1% (w/v) Special Agar Noble. The experiments were performed in gel prepared in phosphate buffers differing in molarity and pH values. The buffer in the apparatus vessels was of the same pH as the buffer in the gel, while its molarity was twice higher. Partially purified virus preparation (conc. 5 mg/ml) was fixed in the presence of 1% formaldehyde at 2°C for a month. It appeared stabilized, *i. e.* did not show electrophoretic heterogeneity present in purfied unfixed TSV preparations and those which were at the initial stage of formaldehyde fixation (Krajačić 1989). The virus was brought into 1.25 mm diameter wells located in a bed of agar cca 2.5 mm high placed at 8×2.5 cm slides. Electrophoresis lasted 4 h under conditions (voltage and amperage) similar to those applied earlier (Rana *et al.* 1987). Following electrophoresis the serum chanals were charged with antiserum to TVS/Cle (homologous titre 1/32, titre to normal proteins 1/4) and serological reactions incubated in a humid atmosphere at room temperature for 24 h.

To distinguish virus precipitin lines from those raised from normal plant proteins, parallel tests with serum absorbed by purified plant proteins were conducted.

Results

Fig. 1 shows that immuno-electrophoretic mobility of TSC/Cle formylated preparation depended on molarity and pH of the buffer. Among three molarities of the buffer in gel (0.008, 0.016 and 0.033 M), at con-

stant pH value of 7.0, the most suitable pattern showed electrophoresis in 0.016 M buffer (Fig. 1b). The use of 0.008 M buffer caused the appearance of a more curved precipitin arc, although electrophoretic movement was considerably smaller (Fig. 1a); in contrast, the migration of virus particles in 0.033 M buffer was the biggest, but the precipitin arc was too much lenghtened (Fig. 1c).

In further experiments the optimal molarity (0.016 M) was combined with some other pH values (6.0, 6.5 and 8.0). As visible from Fig. 1, with raising the pH, electrophoretic movement enlarged, but the precipitin band became more elongated (Fig. 1f). This suggested displacement of TSV from its isoelectric point. Moreover, by diminishing pH below 7.0, an additional precipitin arc from the reaction of normal plant proteins developed (Fig. 1d, e). It was weakly expressed at pH 6.5, and strongly at pH 6.0.

From the results presented it follows that in performing the immuno--electrophoretic experiments with TSV/Cle particles in the conditions used a combination of 0.016 M phosphate buffer and the pH value of 7.0 is appropriate. It proved particularly convenient in observing changes of TSV immuno-electrophoretic pattern during formaldehyde fixation (Krajačić and Štefanac, unpublished results).

Discussion

The influence of buffer on electrophoretic behaviour is well known and logical and rests on the dissociation of carboxyl and amino groups on the protein shell of virus causing alteration of the particle net charge. Some buffers that gave satisfactory results in studies with plant viruses are: 0.025 M acetate-NaCl, pH 5—5.7 (Wagner et Bancroft, 1968); 0.025 M Tris-barbital, pH 8.8 (Van Regenmortel et Lelarge 1973); 0.005 M phosphate, pH 7 and 0.025 M Tris-HCl, pH 8.2 (Paul *et* Querfurth, 1979). The results obtained by Hollings *et* Stone (1975) during investigation of electrophoretic differences among tombusviruses suggest the advantage of phosphate buffer (0.03 M, pH 7.6) over many other buffers of different molarity and pH. Lot and Kaper (1976) prefer the citrate buffer in work with cucumoviruses since, in distinction to phosphate buffer, it prevents their precipitation.

In these experiments the effect of different kinds of buffers was not compared. We decided to use phosphate buffer to ensure electrophoretic medium equal to that employed during study of the influence of formaldehyde on some biophysical and biochemical properties of TSV particles (Krajačić 1989, Krajačić and Štefanac, unpublished results). This buffer medium showed earlier to be adequate in purification of TSV (Lister et Saxena 1976, Rana *et al.* 1987).

Taken in general, all the checked combinations of buffer molarity and pH resulted in eligible immuno-electrophoretic patterns. The purpose of selecting the optimal conditions had for the purpose to obtain well defined separation of all possible electrophoretic components present in virus preparation. As known, the potentiality of the separation is proportional to the velocity of electrophoretic movement and the level of curvature of precipitin arc, and these two qualities alter with changing molarity and buffer pH. By increasing molarity and pH, electrophoretic velocity increases, but less curved precipitin arcs are formed. The results show that in case of TSV/Cle the compromise was a combination of 0.016

M buffer and pH 7.0. Although some other combinations were not checked, we suppose that a good option would also be a half of the selected molarity, i.e. 0.008 M, and pH 8.0.

The appearance of additional immunoprecipitin arc at a pH below 7.0 was not connected with the reaction of disintegrated virus particles. As known, the acid medium (up to pH 5) does not threaten the integrity of TSV particles, the condition used by some authors for clarification of infective plant sap by acidification (Fulton 1967, Lister et Saxena 1976, Rana et al. 1987). The extra electrophoretic arc no doubt originated from normal plant proteins which did not usually react with small quantity of specific antibodies in the serum used. A more acid buffer medium caused partial precipitation of normal proteins resulting in diminishing their antigenicity which became equivalent to the quantity of specific antibodies in the serum.

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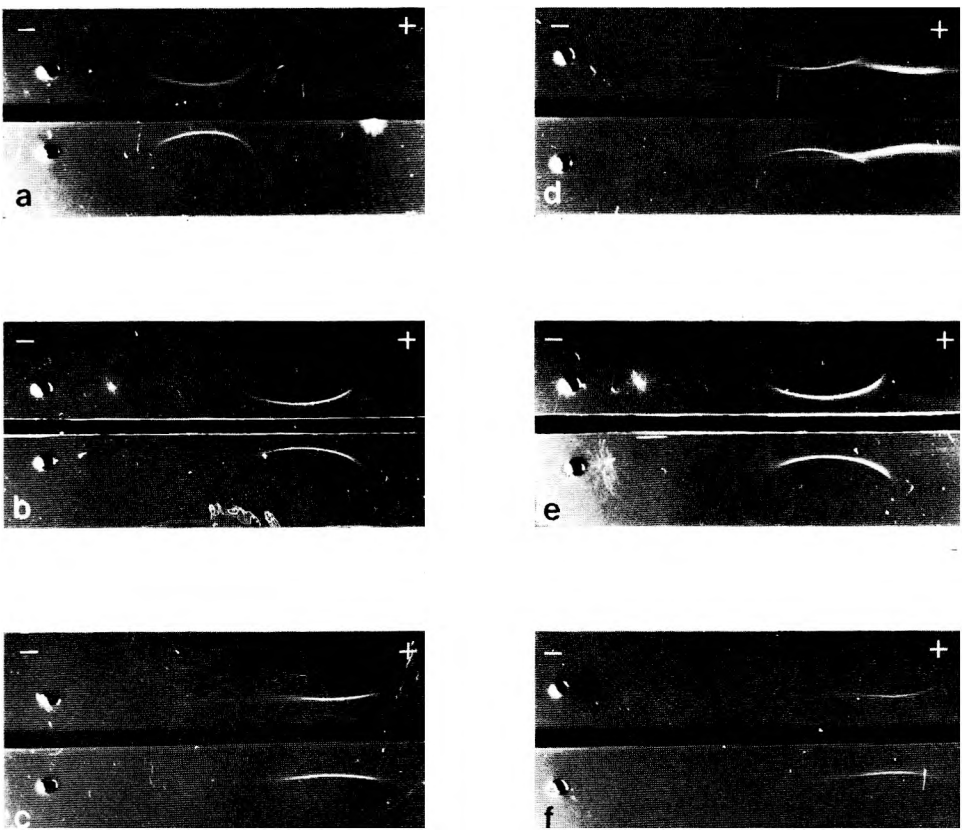

Fig. 1. Immuno-electrophoretic patterns of formylated tobacco streak ilarvirus (TSV-Cle) partially purified preparation (conc. 5 mg/ml) as influenced by the following combinations of phosphate (Sörensen's) gel-buffer molarity and pH: a) 0.008 M, pH 7.0; b) 0.016 M, pH 7.0; c) 0.033 M, pH 7.0; d) 0.016 M, pH 6.0; e) 0.016 M, pH 6.5; f) 0.016 M, pH8.0. Electrophoresis was conducted for 4 h at 2°C, 4 mA and 20 V/cm in 1% (w/v) Special Agar Noble. Original antiserum to TSV/Cle (cf. Materials and Methods) was diluted 1/2.

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SAŽETAK

STANDARDIZIRANJE UVJETA ZA IZVOĐENJE IMUNO-ELEKTROFORETSKIH POKUSA S ČESTICAMA ILARVIRUSA CRTIČAVOSTI DUHANA

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Ustanovljeno je da Sörensenov (natrijev fosfatni) 0,016 M pufer pH 7.0 u agarskom gelu, dvostruko većeg molariteta u bazenima mikroelektroforetskog aparata »LKB-Gelman« 6800 A-1, uz konstantni napon od 20 V/cm gela, jakost struje od 4 mA te izvođenje elektroforeze na temperaturi od 2°C, osigurava optimalnu imunoelektroforetsku sliku čestica ilarvirusa crtičavosti duhana (tobacco streak virus; soj Cle). Navedeni uvjeti udovoljavali su zahtjevima za utvrđivanje elektroforetske heterogenosti nefiksiranih čestica virusa crtičavosti duhana.

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